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THE EFFECT OF SUSPENSION DENSITY ON THE RETENTION OF 5 μM DIATOMS BY THE *MYTILUS EDULIS* GILL[1]

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Wallengren (1905) observed that the latero-frontal cirri, the compound filtering cilia on the gill surface of certain bivalve molluscs (the macrociliobranchia of Atkins, 1938; revised by Owen, 1978), have a series of branches. Carter (1924) and Dral (1967) stated that these branches were produced by degradation and that a healthy cirrus is an unbranched structure that traps particles by adhesion. Recent electron microscope studies (Moore, 1971; Owen, 1974) have verified Wallengren's observation and shown that in *Mytilus edulis*, the distance between branches of the cirrus is 0.6 μm, seemingly with little variation. For the simple case of spherical particles, a perfect retention of 0.6-μm diameter is thus predicted given that a) the branches of cirri act like sieves, b) escape of particles as they impinge on the filter is negligible and c) alternate cirri of the same filament beat synchronously (*i.e.*, regular coordination). Since branches of alternate cirri overlap, condition c), which Owen (1974) considered as "normal," means that the ostial opening is covered by the cirral meshwork at all times. (Only spherical particles are considered in this paper, so that the retention properties of the filter can be characterized by a minimum mesh dimension.)

However, Dral (1967) observed young semitransparent specimens of *M. edulis* in dense algal suspensions and concluded that certain variations in cirral beat could alter the gill's retention efficiency, E%, for particles as large as 20 μm. Although Dral based his estimates on the incorrect assumption that the cirri are unbranched, his observations concerning irregular coordination and halt positions of cirri could still predict a reduced retention of relatively large particles. Since the ostial channel (the gap between adjacent filaments) can be 30- to 40-μm wide, such predictions are theoretically possible. Jorgensen (1975) has indicated that serotonin, by direct action on the positioning of the latero-frontal cirri, may decrease E% for 5-μm yeast cells. Furthermore, Owen (1974) observed that up to two pairs of adjacent cirri on the same filament could beat synchronously and that the activity of the cirri showed "considerable variation." Davids (1964) estimated E% values as low as 20 and 25% for the unicellular algae *Chlorella* sp. and *Isochrysis galbana* (both approximately 5.0-μm diameter). Furthermore, he observed that E% could vary inversely with food concentration.

The present study investigates E% in *Mytilus edulis* at various concentrations of *Thalassiosira pseudonana* (a concentric diatom whose volume, determined by the coulter-counter, equals a sphere of 5-μm diameter) in an attempt to reconcile the above discrepancies between the empirical data and the predictions. In particular, the purposes of the report are a) to re-examine the observations of Davids (1964) and b) to determine if the mechanisms observed by Dral (1967) have any measurable effect on E% for 4.4- to 6.4-μm particles. The discussion will

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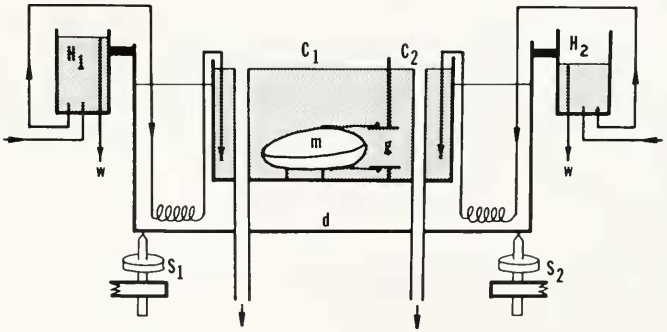

FIGURE 1. Diagrammatic representation of the apparatus used for estimation of gill retention efficiency and pumping rate. Arrows indicate the direction of water flow. W signifies flow to waste from the constant heads. For explanation of other lettering, see text.

include how cirral morphology and function can account for some published observations of E% for various particle sizes.

MATERIALS AND METHODS

Exhaled water was separated from specimens of the mussel, *M. edulis,* by attaching lightweight rubber sleeves to the valves around the exhalant aperture regions. A fuller account of the following description is given by Hildreth (1976).

The apparatus is shown in Figure 1. The water bath, d, is supported on three screws (only two, S_1 and S_2 are shown) which enable the water levels in chambers C_1 and C_2 to be altered with respect to one another. To ensure that the hydrostatic pressure difference between C_1 and C_2 is zero, the following pre-adjustments are made: 1) with connecting pipe g closed, the overflow rates from C_1 and C_2 (supplied by constant heads H_1 and H_2, respectively) are measured; 2) with g open, screws S_1 and S_2 are adjusted so that the overflow rates from C_1 and C_2 are equal to their respective input rates from H_1 and H_2. (H_1 and H_2 supply rates to C_1 and C_2 are 250 and 25 ml/min, respectively.)

The mussel's rubber sleeve is then connected to pipe g so that the mussel, m, inhales from C_1 and exhales into C_2. The mussel's pumping rate, r_b, is estimated as the volume overflow rate from C_2 minus the constant head input from H_2. The overflow rate from C_2 is recorded automatically. Diatom culture is fed into the water supply of H_1.

E% was estimated for 4.4- to 6.4-μm-diameter particles by taking discrete samples simultaneously from the overflows of C_1, C_2, and H_2. Samples were immediately analyzed for particle concentrations (by numbers/ml) using a model Z_B coulter-counter with 140-μm tube. Samples were counted in quadruplicate with a 0.5 ml manometer. The particle concentration of the mussel's exhaled water $[C_2]'$ was calculated as:

$$[C_2]' = ([C_2] - r_a[C_3]/R)R/r_b \qquad (1)$$

where $[C_2]$ is the particle concentration of water leaving C_2, $[C_3]$ is the concentration leaving H_2 (all concentrations in particles/ml), R is the overflow rate from C_2 for the sample's duration and r_a and r_b are the volume input rates to C_2 from H_2 and the mussel, respectively ($r_a + r_b = R$, all flow rates in ml/min).

E% is then calculated as:

$$E\% = (1 - [C_2]'/[C_1]) \times 100 \quad (2)$$

where $[C_1]$ is the particle concentration in chamber C_1. C_1 overflow was also analyzed for 3.4- to 8.4-μm-diameter particles to give what we refer to as food concentration.

Sampling regimes

In regime A, seven mussels were tested individually for 4 hr at each of the following food concentrations (cells/ml): 3.0×10^3, 1.0×10^4, 2.0×10^4, and 9.0×10^4. Estimates of E% were made every 15 min. Pumping rate, R_p (the rate of water flow through the gill in l/hr), was recorded continuously. The order in which feeding concentrations was presented to the mussels was varied.

Four mussels were observed in regime B, which employed food concentrations of: 2.0×10^4, 9.0×10^4, and 1.1×10^5 cells/ml, presented to mussels in that order. Mussels were kept at each concentration for 2 hr during which four to six estimates of E% were made.

Mussels used in this study were collected sublittorally at Spanish Ship Bay, Nova Scotia, Canada. Acclimation to laboratory conditions (15° C) and continuous feeding were ensured. Mussels ranged in length from 4.5 to 6.0 cm.

Errors in E% estimates

Particle concentrations (by numbers or volumes) in a discrete size category can change because of aggregation, and a potential error exists in interpreting such changes as retention by the gill. Although aggregation of healthy, non-colonial diatoms is unlikely (personal observation), its possible occurrence was tested as follows. Water samples like those normally collected to estimate E% were analyzed for their particle size spectra using a model T_{AII} coulter-counter with a 100-μm tube. Particle concentrations (by numbers and volumes) were estimated in 14 size categories for a total size range of 1.78 to 45.3 μm, each category having a mean volume two times larger than the preceding. The test involved several mussels at various feeding concentrations.

A potentially more significant error could result from the resuspension of feces. Hildreth (in press) estimated that a single mussel can produce 10^7 particles/hr (3.4–8.4 μm) when offered algal concentrations (*Phaeodactylum tricornutum* and *Tetraselmis suecica*) at 10^4 cells/ml, and that levels of fecal resuspension and feeding concentration are directly proportional. Of the food types tested by Hildreth (in press), *Thalassiosira pseudonana* gave least contamination in the 3.4- to 8.4-μm size range, and this diatom was used throughout the present study. However, Hildreth (in press) estimated that mussels fed *T. pseudonana* at a feeding concentration of 4×10^4 cells/ml could produce up to 2×10^6 particles/hr. This figure is used in the present report as an approximate correction factor for estimates of E% at feeding concentrations above 4×10^4 cells/ml.

Results

The data collected in regime A (Fig. 2) show that mean gill-retention efficiency (for seven mussels) for 4.4- to 6.4-μm-diameter particles remains virtually constant as suspension density increases, even when mussels produce pseudofeces. Mean

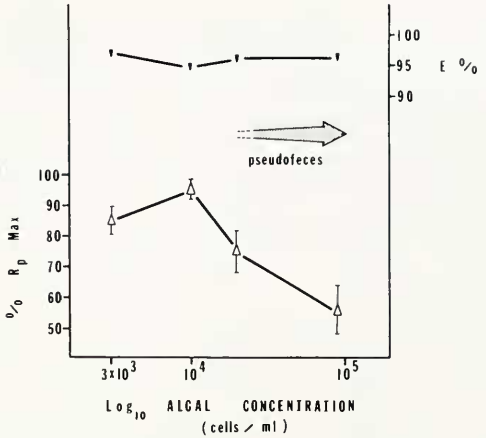

FIGURE 2. Gill retention efficiency (E%) and % maximum pumping rate (% R_p max) as a function of suspension density. Bars on R_p max values are standard errors. Standard errors on E% values are $< 1\%$.

E% from regime B (four mussels) also remained virtually constant with increasing suspension density and was 97.5% at 1.1×10^5 cells/ml. At densities above 10^4 cells/ml, reported E% values have been increased by 1% to compensate for errors introduced by fecal resuspension (see Materials and Methods). Examination of the particle size distributions of inhaled and exhaled water suggested no interference by aggregation.

The decrease in pumping rate, R_p, between cell concentrations of 10^4 and 9×10^4 cells/ml (Fig. 2) is due either to a decreasing fraction of the total ostia being used, an observation made by Foster-Smith (1974), or to a reduction in lateral ciliary activity. The latter possibility could be due to interference with the cilia by increasing quantities of particulate matter in the ostia or, as Rubenstein and Koehl (1977) have pointed out, because the latero-frontal cirri become so clogged that there is an increasing resistance to flow. The mean pumping rate recorded at 10^4 cells/ml was 5.6 l/hr (for a mean dry flesh weight of 0.70 g).

DISCUSSION

The present study shows that retention efficiency of 5.0-μm diatoms for *Mytilus edulis* remains constantly high over a wide range of particle concentrations. This differs from the results of Davids (1964). However, Davids may have incorporated an artifact into his experimental design, since the hydrostatic pressure in his chamber I was greater than in II (symbols from his Figure 1). Thus when the mussel opened, unfiltered water could pass from I to II through any leak in the mussel/sleeve attachment. Hildreth (1976) has shown how such leaks can be of quantitative importance if a hydrostatic imbalance exists between the chambers. In addition, at low suspension densities, the relatively high pumping rate of Davids' mussel would tend to counterbalance the hydrostatic pressure difference between chambers, thus minimizing the flow of unfiltered water from I to II. At high suspension densities, however, a decrease in pumping would have less counter-effect on the flow of unfiltered water, hence an observed inverse relationship between E% and suspension density.

The capacity to vary the retention efficiency of a filtering organ would clearly be of value to a filter feeder. The organism could, for instance, respond passively to high suspended loads in its environment (*i.e.*, by not filtering but still respiring) and minimize the energy expended in rejecting unwanted material. If a filter feeder acts simply to maximize its energy gain from the environment (Lehman, 1976), one would expect an organism which controlled its filter efficiency to possess some advantage over an organism with no control. Taking the argument further, one might expect the effective filter efficiency (*i.e.*, the filtration rate in g of material filtered per unit time) to correlate with optimum ingestion rate. Thus, reported variance in retention efficiencies for a specific size of particle are appealing. However, as Doyle (1979) has pointed out, natural selection may have put constraints on feeding behavior other than those imposed by energy maximization principles. Bivalve filter feeders are clearly an example of this point, since the gill is a complex organ in which the water current is produced within the ostial channel by the simple lateral cilia, but filtration is performed on the gill surface by latero-frontal cirri. An active response to high suspended loads is thus demonstrated by *M. edulis* since, as opposed to a feeding function, the latero-frontal cirri have a major gill-cleansing function in guarding the entrance to the ostial channel.

The gill is the major respiratory organ in the mantle cavity, since it is responsible for producing the ventilation current and probably for much of the gas exchange. The efficiency of oxygen removal for the whole animal is between 5 and 10% under normal conditions but can rise to at least 35% during environmental hypoxia (Bayne, 1971). It would be interesting to see if the efficiency of oxygen removal increases in high suspension densities, an effect perhaps difficult to achieve if the latero-frontal cirri allowed high particulate levels in the ostial channel. Widdows *et al.* (1979), using resuspended, naturally occurring particles, have observed a significant increase in oxygen extraction efficiency with increasing particle concentration. The tips of lateral cilia (15 μm long) of adjacent filaments may touch during their beat cycle (Sleigh, 1974), and so particulate matter in the ostial channel presumably interferes with the coordination and power output. Widening of the ostial channel by muscular and vascular action could act to reduce such interference but probably not by much, according to the dimensions quoted by Dral (1967) and Foster-Smith (1976).

Inferences concerning the coordination of the latero-frontal cirri can be made by comparing E% and R_p for individual mussels that show enough variation in R_p to make this feasible. Figure 3 indicates that the majority of mussels tested exhibit cirral coordination over the range of pumping rates observed.

Moore's (1971) and Owen's (1974) estimate of a minimum mesh dimension of 0.6 μm is only valid if adjacent cirri on the same filament are asynchronous by half a beat. Examination of Owen's (1974) Figure 17 shows that one synchronous pair of cirri would produce a "hole" of approximately 2 μm in the filter meshwork. One and a half or two synchronous pairs would locally increase the minimum mesh dimension to 4 or 6 μm, and so on. The phenomenon of synchrony in adjacent cirri would give a significant variance to the filter's minimum mesh dimension, which could explain the decreasing percentage capture of particles < 2 to 4 μm observed for the macrociliobranchia (Haven and Morales–Alamo, 1970; Vahl, 1973) and also the low retention (about 86%) of 4.4- to 6.4-μm particles occasionally observed in the present study (Fig. 3). Irregular spacing between cirri (Dral, 1967) and a length of 1.4 μm between distal branches of the

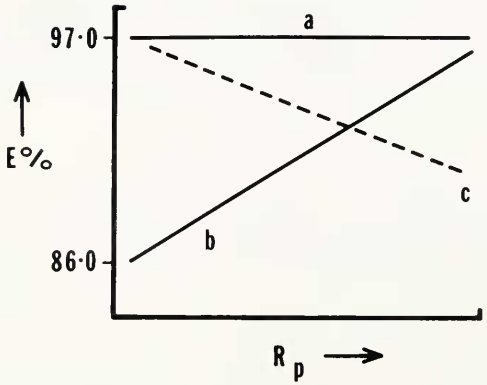

FIGURE 3. Diagrammatic representation of the relationship between E% and R_p, based on individual data for nine mussels fed at 9×10^4 cells/ml. For type a) behavior (shown by six of the mussels tested), latero-frontal cirral activity appears to be coordinated (*i.e.*, alternate cirri are in phase). Types b) and c) behavior (each shown by 1 mussel) indicate degrees of cirral uncoordination (*i.e.*, ≥ 3 adjacent cirri become synchronous or stop beating in a halt position). One mussel showed a b)/c) combination. Types b) and c) behavior were not frequent enough to lower the mean E% values given in Figure 2.

cirrus (Jorgensen, 1975) would also increase the variance in minimum mesh dimension. When considering the whole gill surface, it is probable that water flows preferentially through the larger holes due to decreased drag. Although considered infrequent, synchrony of adjacent cirri could thus cause significant decreases in retention efficiency. Observed retention efficiencies can therefore be explained by the sieving action of the cirri, and the alternate mechanisms of particle capture proposed for mussels by Rubenstein and Koehl (1977) may not apply. The situation here is somewhat analagous to that commented on by Boyd (1976). Boyd suggests that reported retention efficiencies of copepods' second maxillae are a function of the significant variance in mean intersetule distance. Reported retention efficiencies for copepods can then be explained by the physical properties of the filter mesh rather than by particle size-selection behavior by the animal.

The observations of Jorgensen (1975) concerning the effects of serotonin solutions on the porosity of the gill are difficult to interpret with respect to the natural situation. Many workers assume 100% retention efficiency of circa-5-μm particles in order to estimate filtration rates. And many have claimed to have shown 100% retention of such particles. Although we observed a constant high retention efficiency, further investigation is clearly necessary. Care in interpretation of retention observations should be exercised, however, because *M. edulis* is known to give rise to bioseston (Vahl, 1972; Hildreth, in press); and escape of smaller particles is likely since cirral branches have stiffening rods which extend distally less than half way up their length (Owen, 1974).

For 4.4- to 6.4-μm particles, the observations made by Dral (1967) concerning *Mytilus edulis* are not as quantitatively significant as he supposed. We conclude that the continuous meshwork formed by the latero-frontal cirri does not allow the gill to become porous to this particle size, even at high suspension densities when the rejection of filtered material could become energetically costly. It seems to be more expedient for a mussel to keep its ostia clear. However, the variations in

cirral beat described by Dral (1967) and Owen (1974) could explain the empirical data on the gill's retention efficiency for smaller particles than were examined in the present report. It is intended that this aspect will be the basis for a further study.

Summary

1. The retention efficiency, E%, of the *Mytilus edulis* gill for 5.0-μm diatoms (*Thalassiosira pseudonana*) was estimated by separating the bivalve's exhalant current through a rubber sleeve attached to the valves around the exhalant siphon.

2. The particle concentration in the 4.4– to 6.4-μm size range of inhaled and exhaled water was estimated with a model Z_B coulter-counter. The mussel's pumping rate, R_p, was recorded automatically.

3. E% and R_p were observed for 11 mussels at diatom concentrations ranging from 3×10^3 to 1.1×10^5 cells/ml. R_p was maximal at 10^4 cells/ml and at 9.0×10^4 cells/ml decreased to about 55% of its maximum value. E% remained constantly high (about 97%) over the range of concentrations even when pseudofeces (filtered material rejected before ingestion) were produced. This result supports the argument that, in general, a continuous filtering meshwork is formed over the ostia by synchrony in alternate (or alternate pairs of) latero-frontal cirri on the same gill filament.

4. Occasionally, a low E% value (about 86%) was observed for an individual mussel and this was attributed to synchrony in $\geqslant 3$ adjacent cirri.

5. Synchrony in one or more pairs of adjacent cirri is advanced as an explanation for published data on the retention of different sized particles.

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